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STUDIES ON THE BIOCHEMISTRY OF SULPHUR

VIII. THE RATE OF ABSORPTION OF CYSTINE FROM THE GASTROINTESTINAL TRACT OF THE WHITE RAT

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VIII. THE RATE OF ABSORPTION OF CYSTINE FROM THE GASTROINTESTINAL TRACT OF THE WHITE RAT

It is now well recognized that a study of sulphur metabolism, in particular the study of cystine, is of prime importance in the understanding of nutrition, cellular respiration, oxidation and reduction, and biochemical defense. In addition to the knowledge that cystine is essential for proper maintenance and growth, that it constitutes a large part of the skin, hair, and nails, and has at times been reported present in relatively large amounts in the abnormal condition known as cystinuria, two recent discoveries have shown that cystine has certain other specific functions in body economy. These discoveries are the isolation by Hopkins (1921, 1929) of the cystine or cysteine complex, glutathione, important in oxidation reduction and in cellular respiration, and the finding by du Vigneaud (1927) that cystine is a vital part of Abel's (1927) crystalline insulin, the pancreatic hormone regulating sugar metabolism.

In view of the manifold functions of cystine, directly or indirectly, in life activities, it is obvious that every bit of detailed knowledge acquired as to the course of its metabolism, especially its rate of absorption and the change it undergoes, is decidedly worth while.

Considerable work has been done, in fact, on cystine absorption by following the change in urea, amino nitrogen, sulphur, etc., in blood and urine after cystine feeding or injection. Such studies were made by Blum (1904), Levene and Kober (1909), Levene and Meyer (1909), Wolf and Österberg (1912), Lewis and Root (1922), Seth and Luck (1925), and Stearns and Lewis (1930).

Cystine, however, has a course somewhat different from that of the ordinary amino acid in that among other pathways it is in part converted to glutathione, in part to taurocholic acid, and is also tied in complexes leading to the formation of keratin, such as exists in the hair, nails, and other epidermal tissues. It would seem from the complexity of the pathways of cystine metabolism that a more definite method of determining the rate of absorption of cystine would be that employed by Stearns and Lewis (1930) and Wilson (1930), following the procedure employed by Cori (1925) for sugar and certain amino acids. In the work of Stearns and Lewis with rabbits, and that of Wilson with the white rat, a known amount of cystine was fed by stomach tube and after a given time the animal was killed and the

amount of substance remaining in the gastrointestinal tract was determined. Stearns and Lewis concluded that the absorption of cystine fed as the sodium salt was not very rapid, since, after six hours, there was found in the gastrointestinal tract of the two rabbits so fed, 20 and 33 per cent of the amount of cystine fed, as determined in the extract by the Folin-Looney method (1922).

Wilson worked with a large number of rats, and it is in his results that the main interest lies. He concluded that the rate of absorption of cystine by the white rat was 30.5 milligrams of cystine per 100 grams of body weight per hour, a value lower than that for other amino acids reported by Cori (1926–27) and Wilson and Lewis (1929). This conclusion was arrived at by feeding cystine principally as the sodium salt and then determining the cystine left in the gastrointestinal tract two hours later by means of the colorimetric method of Folin and Marenzi (1929). The difference between the quantity of cystine fed and that found in the gastrointestinal tract was made the basis of the determination of the rate of cystine absorption.

Wilson's work seems to be an excellent, well-balanced contribution to the knowledge of the metabolism of this highly important sulphur-containing amino acid, cystine. The only question is his interpretation of his findings with the use of the Folin-Marenzi method. This method is excellent when applied to pure cystine, but as shown in various publications from this laboratory (Sullivan 1929, Sullivan and Hess 1929) it has the disadvantage of reacting with other sulphur compounds and even with compounds not containing sulphur. We have found it, in fact, as good a reagent for glyoxal as for cystine (Sullivan and Hess 1930). The same objection applies to the Folin-Looney method as used by Stearns and Lewis.

Prompted by the work of Wilson, we repeated his cystine work first to see whether the Sullivan method for cystine and the Folin-Marenzi method would agree as to the rate of absorption of cystine from the gastrointestinal tract of the white rat, and we checked the results obtained by these methods with the use of the Okuda (1925) iodometric method. The results verify Wilson's general findings with the Folin-Marenzi method for cystine but not necessarily his interpretation of these findings. In addition the results obtained by the three methods throw some further light on the metabolism of cystine by the white rat.

EXPERIMENTAL

The cystine was weighed out, 130 milligrams for each 100 grams body weight, and suspended in 2 cubic centimeters of distilled water. The theoretical amount of sodium hydroxide was then added dropwise with stirring until all the cystine was in solution.

Feeding experiments.—The freshly made solution of sodium cystinate was fed slowly through a small stomach tube, followed by 1 cubic centimeter of water. The tube was protected by a perforated wooden bar to keep the jaws apart. Four rats were fed cystine and two rats with no feeding were used as controls. All the rats were from the stock colony employed by Passed Asst. Surg. W. H. Sebrell in nutrition studies. The controls and two rats (numbered 3 and 4) fed cystine, were on the same diet. The other two rats, fed cystine (numbered 5 and 6) were on a quantitatively different diet, but the results obtained were of the same order. All had been fasting 24 hours at the time of feeding.

For the absorption studies, a period of two hours was employed. Then the animals were killed by a blow on the head and, after ligation of the esophagus and rectum, the gastrointestinal tract was removed as done by Wilson. At the same time the entire liver was removed for glutathione determinations subsequently detailed.

From this point the procedure differed from that of Wilson. He washed out the tract with a large volume of water, acidified the extract with acetic acid and boiled to destroy proteolytic enzymes and coagulate protein, filtered, and precipitated the filtrate with sodium tungstate. Following the lead of Okuda (1929), who found sulphosalicylic acid a very satisfactory protein precipitant when studying the sulphydryl content of tissue, advantage was taken of sulphosalicylic acid as a protein precipitant. In previous work in this laboratory it was found that sulphosalicylic acid has a definite solvent action on cystine and does not interfere with the estimation of cystine when compared with cystine in 0.1 N hydrochloric acid. Accordingly, the entire gastrointestinal tract was ground with 8 to 10 grams of sand and 30 cubic centimeters of N sulphosalicylic acid (0.5 molar solution). The mixture was filtered through hard paper on a 3-inch Buchner funnel by suction. The sand and tissue débris were then washed with 10 cubic centimeters of N sulphosalicylic acid, and the mixture poured into the Buchner funnel. This procedure, previously tested out in other work, gave 100 per cent recovery of cystine added to liver pulp. It might have been better to have made a second extraction, but the desire was to keep the volume down so that cysteine, if present in the extracts in small amounts, could be satisfactorily determined, and also that determination might be made of small amounts of cystine or cystine derivatives in the extract of the fasting rats not fed cystine. The extract was difficult to filter. When about 15 cubic centimeters had filtered through, 10 cubic centimeters were taken for analysis and the filtering was continued to get the total volume of extract. These 10 cubic centimeters of somewhat opalescent solution taken, were brought to pH 3.5 by means of 5 N sodium hydroxide added dropwise with stirring. For the extract of the

gastrointestinal tract of the fasting rat, without cystine, no further dilution was made. For the extract of the cystine-fed rat the solution at pH 3.5 was made to 100 cubic centimeters with 0.1 N hydrochloric acid.

The results obtained on the cystine content of the fasting rats as such and of fasting rats fed cystine, are given in Tables 1 and 2, without correction for the change in sodium cystinate or correction on the method of extraction. These corrections which would make about 10 per cent variation in the results, will be discussed later.

TABLE 1.—*Cystine or cystine equivalents in the gastrointestinal tract of fasting rats*

Rat No.	Sex	Weight	Cystine			Cystine per 100 grams body weight		
			Sullivan method	Okuda method	Folin-Marenzi method	Sullivan	Okuda	Folin-Marenzi
		Grams	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
1	M	204	<0.4	3.6	5.92	----------	1.76	2.90
2	M	184	<0.4	3.6	7.16	----------	1.96	3.89
Average	------	194	<0.4	3.6	6.54	----------	1.86	3.40

TABLE 2.—*Rate of absorption of cystine fed as sodium salt*

Rat No.	Sex	Weight	Absorption time	Cystine fed per 100 grams body weight	Cystine recovered per 100 grams, corrected for control			Rate of absorption 100 grams body weight per hour		
					Sullivan	Okuda	Folin-Marenzi	Sullivan	Okuda	Folin-Marenzi
		Grams	*Hours*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
3	M	205	2	130	26	29	67	52	51	32
4	M	200	2	130	24	26	79	53	52	26
5	M	180	2	130	20	25	66	55	53	32
6	M	192	2	130	25	30	63	53	50	34
Average	------	194	--------	130	23.8 (22.1)	27.5	68.8	53.3 (54)	51.5	31

Cysteine in extract of gastrointestinal tract.—While the extracts of the gastrointestinal tract of the rats fed cystine were being tested for total cystine—that is, for cystine and cysteine as given under Table 2—they were likewise analyzed for cysteine. As is shown in Table 3, a large amount of the material calculated as cystine left in the gastrointestinal tract of the rats fed cystine is in the reduced form, cysteine. The extract of the tract of rat No. 3 was otherwise used up before the desirability of testing for cysteine arose. In only one case was a determination made for cysteine—that is, without sulphite—by the Folin-Marenzi method, since this application is difficult in extracts of tissue which might contain material similarly reacting, as, for instance, uric acid and ergothionine.

TABLE 3.—*Cysteine content of the gastrointestinal tract of the white rat fed cystine*

Rat	Sex	Weight	Cysteine per 100 grams body weight		
			Sullivan	Okuda	Folin-Marenzi
		Grams	*Milligrams*	*Milligrams*	*Milligrams*
4	M	200	8.6	9.6	
5	M	180	8.6	11.3	
6	M	192	9	11.8	12 7
Average			8.7	10.5	

Of the total material calculated as cystine left in the gastrointestinal tract at the end of two hours as given in Table 2 (rats 4, 5, and 6), the cysteine found as given in Table 3 is in percentage by the two methods: Sullivan 37.8, Okuda 38.9. The one case tested by the Folin-Marenzi method, without sulphite, gave cysteine 20.1 per cent of the total cystine. The Okuda method gives slightly higher values for cysteine than the Sullivan method because it reacts with other sulphur compounds, such as glutathione, to which the Sullivan reaction is negative.

The relatively high cysteine content found is not in agreement with the work of Wilson. He noted that a certain amount of cysteine, mercaptan, or sulphides occurred in the extract of the gastrointestinal tract of white rats, especially after feeding cystine. This he neglected, since the amount found at the end of two hours was estimated to be equivalent to only 2 or 3 milligrams of cystine. In view of the known reducing capacity of living tissue, this amount seems small and is probably small in Wilson's experiment because of the procedure employed for deproteinizing, heating, etc., since cysteine, under the conditions, tends to oxidize to cystine on exposure to air and heat.

The presence of cysteine calls for a correction on the total cystine findings, especially in the Sullivan method. On the average, cystine gives about 80 per cent as much color as cysteine. The correction[1] based on the cysteine is applied to the average figures of Table 2 and given in parentheses. Subsequently corrections will be made for the cystine content of the sodium cystinate fed and for the efficiency of the method of extraction.

The data given in Table 1, on the cystine equivalent of the reducing substance in the gastrointestinal tract of the fasting rat as determined by the Folin-Marenzi method, is very much smaller than that given by Wilson. On account of the small volume of the sulphosaliylic acid extract, direct comparison with a 200 part per million cystine standard was possible in this method. We have found

[1] The best procedure in dealing with a mixture of cystine and cysteine is to oxidize the cysteine with a current of air before matching colorimetrically with a cystine standard.

direct comparison much more accurate than determination by difference—that is, comparison of a known cystine solution with a similar solution plus the unknown—especially where a large multiplication factor is involved in the method of difference. The intestines of the fasting rats showed practically no free cystine by the Sullivan method and only a small amount of total glutathione or other disulphides by the Okuda method, and likewise only a small amount of reducing material by the Folin-Marenzi method.

The data given in Table 2 for the Folin-Marenzi method agree with the findings of Wilson in giving the rate of absorption of cystine fed as the sodium salt as 31. The Sullivan and Okuda methods give a much higher absorption rate. This difference calls for interpretation of results.

The Sullivan method is a method of the greatest specificity for cysteine and for cystine when reduced before beginning the test. For its positive outcome it requires that the three groups SH, NH_2, and COOH be free and in the order as existing in cysteine, since isocysteine is negative (Sullivan and Hess (1930)). The drawback in the Sullivan method is that a mixture of cystine and cysteine will give slightly higher color readings than an equivalent amount of cystine. The Okuda iodometric method has been found in work previously reported, Sullivan (1929) and Sullivan and Hess (1929), to give a positive reaction with various sulphur compounds such as contain the (SH) or (S–S) grouping. The Folin-Marenzi method has likewise been found to give positive reactions with the same sulphur compounds as the Okuda method and also with certain nonsulphur compounds of high reducing capacity. As previously stated, it is, in the presence of sodium sulphite, as good a reagent for glyoxal as for cystine. In short, the Sullivan method is the only method with a high degree of specificity for cysteine or cystine.

In the light of these facts, the agreement between the Okuda method and the Sullivan method, both of which disagree with the findings by the Folin-Marenzi method, shows that the Folin-Marenzi method is giving a positive reaction with some nonsulphur containing complex in the gastrointestinal tract of the rats fed cystine. Since the extract of the gastrointestinal tract of the fasting rat, without cystine, shows little reducing capacity in the Folin-Marenzi method, the desulphurized reducing material in the tract of the rats fed cystine may be changed cystine. If this is so it is possible that the rate of absorption of cystine by the white rat is as found by the Folin-Marenzi method. A corollary of this conclusion is the important finding that desulphurization occurs speedily in the intestinal tract, since a comparison of the results, given by the Okuda method and the Folin-Marenzi method, indicates that over 30 per cent (31.77) of the cystine fed has been left in the gastrointestinal tract as a non-

sulphur compound reacting only with the Folin-Marenzi method. An analysis of the results indicates also that only about 4 per cent of the cystine fed is left in the tract as a deaminized cystine, or possibly a decarboxylated cystine.

A second possibility, however, is that the excess of reducing material found in the gastrointestinal tract by the Folin-Marenzi method as compared with the Sullivan and the Okuda methods, has nothing to do with cystine or changed cystine but is merely highly reducing nonsulphur containing material, digestive or otherwise, secreted by the tract under the stimulus of the sodium cystinate fed.

Attention was given to the problem of explaining the divergence of our findings from those of Wilson, who used the one method, Folin-Marenzi, for cystine studies. The task led to a study of (1) the stability of cystine when made into a sodium salt, (2) the efficiency of extraction of cystine by sulphosalicylic acid, (3) the change in cystine added to the isolated intestines, and (4) the reducing capacity of the gastrointestinal tract of rats fed amino acids, such as glycine and alanine. These points may be taken up separately.

The stability of cystine in sodium cystinate.—By care, cystine can be converted to the sodium salt in a small volume of solution without appreciable injury to the cystine. Theoretically and actually the quick addition of the alkali may make a change in the cystine leading to more or less deaminization. This deaminized product would react like cystine in the Folin-Marenzi and in the Okuda methods but not in the Sullivan method.[2] Since the question of the stability of the sodium cystinate was considered important, the details on this point may be given. Thus 250 milligrams of cystine were suspended in 2 cubic centimeters of water and to the mixture were added dropwise (0.05 cubic centimeter at a time) with stirring, 2.1 cubic centimeters of N sodium hydroxide. The solution was then made to 25 cubic centimeters with water to make a stock solution, each cubic centimeter of which contained 10 milligrams of cystine.

For the Okuda method 1 cubic centimeter was treated with 1 cubic centimeter of 0.09 N hydrochloric acid, to overbalance slightly the sodium present. Then 2 cubic centimeters of 20 per cent hydrochloric acid were added and the whole was made to 20 cubic centimeters. This solution was matched in the Okuda iodometric cystine method against 10 milligrams of cystine in 2 per cent hydrochloric acid. Both solutions were reduced by heating with zinc powder for 10 minutes.

For the Sullivan and Folin-Marenzi methods 1 cubic centimeter of the stock sodium cystinate containing 10 milligrams of cystine was treated with 1 cubic centimeter of 0.09 N hydrochloric acid and 2 cubic centimeters of 0.2 N acid and made to 50 cubic centimeters with

[2] Cystine deaminized by the method of Neuberg and Ascher (1907) is negative in the Sullivan reaction and positive in the Okuda and the Folin-Marenzi methods.

0.1 N hydrochloric acid. Of this solution, 5 cubic centimeters containing 1 milligram of cystine was matched against 5 cubic centimeters of a 200 part per million cystine in 0.1 N hydrochloric acid.

Tested at once, in 2 hours, and 24 hours, the stock sodium cystinate solution showed no change in the cystine content as measured by the Sullivan reaction or in the other methods. Even after standing one week in a closed flask at room temperature there was little, if any, change from the theoretical cystine content.

The early finding that a stable solution of sodium cystinate could be made if reasonable care were employed, coupled with the fact that previous investigators, in so far as their published work shows, did not consider the possibility of change in the cystine in making the sodium salt, led to the idea that this point was settled. However, in going over points to be controlled before publication of the results, several samples of sodium cystinate were made and the indication was noted that care must be exercised in the neutralization with alkali if deaminization is to be avoided. The results of this work are shown in Table 4.

TABLE 4.—*The cystine content of sodium cystinate*

Solution	Cystine weighed	Water used	N NaOH used	Cystine found in sodium salt		
				Sullivan	Folin-Marenzi	Okuda
	Mg.	*C. c.*	*C. c.*	*Per cent*	*Per cent*	*Per cent*
A	250	2	2.10	100	100	98.5
B	50	1	.43	89	100	98.3
C	600	5	5.20	95	97	100.0
Average				94.7	99	98.9

For the cystine determinations, the solutions of sodium cystinate were diluted to appropriate volumes with water and used as such or in aliquots in comparison with a 400 part per million cystine in 0.1 N hydrochloric acid.

The Sullivan method indicates that it is possible to have some deaminization in making the sodium salt.

Recovery of cystine by method of extraction.—For the determination of the efficiency of extraction, solution C of Table 4 was employed and diluted with water to 12 cubic centimeters. Four cubic centimeters, corresponding to 200 milligrams of cystine weighed out but analyzing 95 per cent of the theory in the Sullivan method, were mixed with the entire gastrointestinal tract of a fasting rat weighing 150 grams and ground with sand and sulphosalicylic acid as in the absorption studies given in Table 2. Analysis of the extract by the three methods gives the recovery of cystine in the method of extraction employed. The results corrected for the cystine content of the gastrointestinal tract

of the unfed rat and for the cystine value of the sodium cystinate used are given in Table 5.

TABLE 5.—*Recovery of cystine by method of extraction. (Cystine ground with gastrointestinal tract, sand, and sulphosalicylic acid)*

Cystine method	Cystine in sodium cystinate used	Cystine in extract corrected for Table 1	Per cent recovery
	Milligrams	*Milligrams*	
Sullivan	190	171.00	90
Folin-Marenzi	194	182.91	94.3
Okuda	200	182.70	91.4

The recovery by the method of extraction of cystine ground with the gastrointestinal tract of the unfed rat was found to be 90 per cent by the Sullivan method, 94.3 per cent by the Folin-Marenzi method, and 91.4 per cent by the Okuda method, all practically of the same order of magnitude.

With the data gathered on the presence of cysteine in the extract of the gastrointestinal tract, the cystine value of the sodium cystinate fed, and the recovery by the method used, the approximately true rate of absorption can be determined. This has been done with the results given in Table 6.

TABLE 6.—*Rate of absorption of cystine (corrected)*

Cystine absorbed or destroyed per 100 grams body weight per hour		
Sullivan method	Okuda method	Folin-Marenzi method
Milligrams 49.33	*Milligrams* 49.24	*Milligrams* 27.85

As previously pointed out, the difference between the results by the Folin-Marenzi method and the other two methods lies either in the presence of a desulphurized complex from cystine reactive only to the Folin-Marenzi method or in the presence of reducing material secreted by the tract under the stimulus of the cystine added. A solution of the dilemma was attempted by a study of the action of the isolated but intact gut on cystine, and the effect of feeding other amino acids on the apparent cystine content of the extract of the gastrointestinal tract.

The change in cystine in contact with the intestines.—An attempt was made to place approximately 200 milligrams of cystine in the isolated gastrointestinal tract of a female rat weighing 150 grams. It was found unsatisfactory to introduce the sodium cystinate into the cut esophagus. Accordingly, for convenience, only the intestines

from the stomach to the rectum were used in contact studies. The rectal end was tied and into the duodenal end was put 4 cubic centimeters of sodium cystinate solution, equivalent to 200 milligrams of cystine as weighed out. The duodenal end was then ligated. The intestines and contents and the stomach and esophagus were placed in a mortar and put into an incubator at 37.5° C. for two hours. Then the whole was ground in the same mortar with sand and 40 cubic centimeters of sulphosalicylic acid in the usual way and filtered. Of the filtrate, 5 cubic centimeters brought to pH 3.5 were diluted to 50 cubic centimeters with 0.1 N hydrochloric acid. Then estimation of cystine and cysteine was made. The results, with corrections, for the cystine in the sodium cystinate, by the respective methods (Table 4, exp. 3) and for the return on the method of extraction (Table 5), are given in Table 7.

TABLE 7.—*The effect of incubating cystine with intestines (percentage recovery of cystine)*

Cystine method	Cystine implanted	Cystine recovered, corrected for findings in Table 1	Cystine recovered, corrected for return on method of extraction	Per cent recovery of cystine
	Milligrams	*Milligrams*	*Milligrams*	
Sullivan	190	163.20 (161.52)	181.33 (179.47)	95.44 (94.46)
Folin-Marenzi	194	172.66	183.10	94.38
Okuda	200	170.80	186.87	93.44

As determined by the Okuda method, without reduction, the extract contained 12 milligrams of reduced sulphydryl compounds. In the fasting rat not fed cystine, as given in Table 1, there were 3.6 milligrams of material comparable to glutathione. The difference, 8.4 milligrams, would represent cysteine found in the two hours' incubation. A correction for this amount of cysteine is made in the Sullivan method in the figures in parentheses.

The data in Table 6 give approximately the same value for all three methods and indicate that there is not much change in the cystine by two hours' contact with the intestines at 37.5° C. The small amount of change, approximately 6 per cent, if real, indicates desulphurization rather than deaminization. The finding of little difference in cystine by incubation in this way with the intestines might be taken to indicate that the difference in results on the rate of absorption of cystine by the three methods as given in Table 2 is due to nonsulphur-containing fluid secreted by the tract under the stimulus of sodium cystinate.

This hypothesis is difficult to prove, but some evidence is at hand in favor of it. Thus, as shown in Table 3, a large amount of the

material calculated as cystine left in the gastrointestinal tract of the rats fed cystine is in the form of cysteine.

The presence of cysteine indicates a high reducing capacity in the gastrointestinal tract. The possibility that it resides in juice secreted by the tract was tested by feeding other amino acids, not containing sulphur, in molecular proportions to the cystine fed. Thus glycine and alanine, converted to the sodium salts, were fed to rats, and at the end of two hours, with all conditions kept as in the cystine feeding, tests were made for cystine in the extract by the Folin-Marenzi method. The results are given in Table 8.

TABLE 8.—*Cystine equivalent of the extract of the gastrointestinal tract of rats fed glycine and alanine (Folin-Marenzi cystine method)*

Rat	Sex	Weight	Absorption time	Substance fed	Amount fed per 100 gm. body weight	Cystine found per 100 gm. body weight	Increase per 100 gm. body weight over fasting rat
		Grams	*Hours*		*Milligrams*	*Milligrams*	*Milligrams*
7	F	155	2	Glycine	81.2	8.4	5.0
8	F	162	2	Alanine	96.4	16.0	12.6

The data given in Table 8 show that glycine to a slight degree and alanine to a considerable degree give extracts responding positively for cystine by the Folin-Marenzi method. With the Sullivan method the cystine test was practically negative, and with the Okuda method it agreed with the results obtained on the fasting rat without amino acid addition.

It would seem that part, at least, of the excess reducing material found by the Folin-Marenzi method over that found by the Sullivan and the Okuda methods is due to material secreted by the intestinal tract under the stimulus of added amino acids. Most of the excess of residual cystine found by the Folin-Marenzi method, however, seems to be due either to a changed desulphurized cystine or to potent reducing material secreted by the tract under the stimulus of the cystine fed and leaving a residue in the tract with no direct relation to cystine.

In the following section it is shown that it is possible to get a positive Folin-Marenzi cystine reaction without any possibility of cystine being the causal agent.

Cystine equivalent of hydrolysates of amino acids, with no cystine or sulphur compound present.—In other work in this laboratory it was found that a mixture of amino acids containing no cystine will, on hydrolysis with acid, yield a fluid with a relatively high cystine equivalent as determined by the Folin-Marenzi method, though absolutely negative in the Sullivan method and the Okuda method.

Thus, based on the percentage of amino acids found in casein as given by Hawk and Bergeim's Practical Physiological Chemistry (9th ed., p. 118), a mixture was made of amino acids readily available. Cystine, for obvious reasons, was left out. This mixture consisted of glycine 4.5 milligrams, alanine 18.5 milligrams, valine 79.3 milligrams, leucine 97 milligrams, proline 76 milligrams, phenylalanine 38.8 milligrams, aspartic acid 41 milligrams, glutamic acid 27 milligrams, hydroxyglutamic acid 51 milligrams, tyrosine 65 milligrams, arginine 38 milligrams, lysine 76 milligrams, histidine 28 milligrams, and tryptophane 22 milligrams, or a total of 662 milligrams of amino acids was hydrolyzed for 6 hours with 10 cubic centimeters of 20 per cent hydrochloric acid in an oil bath at 120 to 125° C. The hydrolysate was decolorized with 60 milligrams of carboraffin and brought to pH 3.5 by means of 5 N sodium hydroxide added dropwise with stirring and made to 25 cubic centimeters with 0.1 N hydrochloric acid. This solution on the weight of the amino acids used, had a cystine equivalent in the Folin-Marenzi method of 0.98 per cent cystine, and was absolutely negative in the Sullivan and the Okuda procedures. It is obvious from this that the Folin-Marenzi method is not necessarily a test for cystine or sulphur compounds, or even for changed cystine. In justice to the Folin-Marenzi method, however, it may be said that in previous work (Sullivan and Hess, 1930) it gave approximately the same cystine content as did the Sullivan and the Okuda methods for serumalbumin and wool.

In the present study, despite the objections which may be raised against the Folin-Marenzi method, the positive cystine reaction with hydrolysates of amino acids with no cystine or sulphur compound present, the positive reaction after feeding glycine and alanine, the positive reaction with glyoxals, in short, its lack of specificity, it is possible, with the acceptation of one main assumption, which will be detailed presently, that this method gives the true rate of absorption of cystine by the white rat. It is certain that the increased cystine value of the extract of the gastrointestinal tract is tied up with the feeding of cystine. Assuming that this excess of cystine value found in the gastrointestinal tract is due to changed cystine to which the other methods are nonreactive, then the rate of absorption of cystine is approximately that found by Wilson—somewhere around 30 milligrams per 100 grams of body weight of rat per hour.

The other alternative is that the value of the rate of absorption by the Folin-Marenzi method is too low and that on the contrary the findings by the Sullivan and the Okuda methods, in round numbers 50 milligrams per 100 grams of body weight per hour, is the correct answer. Neither of these methods gives any cystine value in hydrolysates of amino acids free from cystine, neither gives any cystine value to extracts of gastrointestinal tract of rats fed glycine or

alanine. If these facts are taken as an indication that these entirely different methods, the Sullivan and the Okuda, which give closely agreeing results, are the true values for the rate of absorption of cystine, then cystine absorption falls in line with that of the other amino acids fed as the sodium salts by Wilson and Lewis (1930). It is absorbed as well as d, l-alanine, better than leucine, and less than glycine or glutamic acid. The final answer is yet to be ascertained.

Glutathione increase in the liver of rats fed cystine.—Since one of the pathways of utilization of cystine absorbed might be through the formation of glutathione, a study was made of the livers of the rats fed cystine, with the fasting rats and the rats fed glycine and alanine as controls. In work with Passed Asst. Surg. W. H. Sebrell on the effect of feeding cystine and other sulphur compounds on the economy of the white rat, as yet unpublished, the adding of cystine to a diet containing casein as the sole protein was found to increase the glutathione content of the liver. This fact was established by the determination of total (S–S) compounds or total (SH) compounds by means of the Okuda iodometric method in conjunction with the Sullivan method, which showed the cysteine or cystine content of the livers to be negligible. In the present work the entire liver was ground with approximately 5 grams of sand and 15 to 20 cubic centimeters of N sulphosalicylic acid as recommended by Okuda. The procedure followed is that recommended by Okuda (1929) for cystine with reduction of (S–S) compounds to (SH) by heating the extract, made 2 per cent acid with hydrochloric acid, with zinc powder for 10 to 15 minutes and titrating the (SH) compounds with M/500 KIO_3 in the presence of KI. Comparisons were made with results of the same method applied to the liver of fasting rats and of nonfasting rats and with the results obtained by Thompson and Voegtlin (1926) for the liver of rats of comparable weights, by means of the Tunnicliffe (1925) method. The findings are given in Table 9.

TABLE 9.—*The glutathione content of the liver of white rats fed cystine, glycine, and alanine*

Rat No.	Sex	Weight	Amino acid fed	Amount fed	Weight of liver	Total glutathione in liver
		Gms.		*Mgs.*	*Gms.*	*Per cent*
3	M	205	Cystine	267	8	0.24
4	M	200	do	260	8	.25
5	M	180	do	234	8	.24
6	M	192	do	250	7.8	.23
7	F	155	Glycine	126	8	.15
8	F	162	Alanine	154	8	.15
Average glutathione of liver of rats weighing 142 to 206 gms. (Thompson and Voegtlin)						.178
Average glutathione of liver of nonfasting rats found in this laboratory						.18

The data given in Table 9 indicate an increase in total combined (S–S) compounds calculated as glutathione over the average found for the liver of nonfasting rats and still greater over the rats fed glycine and alanine in using the Okuda method and over the results found by Thompson and Voegtlin (1926) for the liver of rats of comparable weight using the Tunnicliffe (1925) method. Thompson and Voegtlin found the average glutathione content of the entire rat to be 23 milligrams per 100 grams of tissue for rats weighing 137 to 170 grams. Accordingly, if the increase in the glutathione content of the liver noted in the present study should be carried over to the entire animal it would explain only a small part of the cystine absorbed as determined by the Sullivan or Okuda methods and also by the Folin-Marenzi method.

The interesting point, however, is that under cystine feeding the glutathione content of the liver was found increased.

SUMMARY

Studies were made on the rate of absorption of cystine, fed as the sodium salt, from the gastrointestinal tract of the rat by means of three cystine methods, the Folin-Marenzi method, the Okuda iodometric method, and the Sullivan method.

The Sullivan and the Okuda methods are in close agreement when corrections are made for the cystine value of the sodium cystinate fed and the percentage recovery of cystine in the method of extraction. The value found by these methods is, in round numbers, 50 milligrams of cystine per 100 grams of body weight per hour—a value which puts cystine in the same class as other amino acids as far as absorption is concerned.

The Folin-Marenzi method gives a lower value, in round numbers, 30 milligrams of cystine per 100 grams of body weight per hour, in agreement with the work of Wilson with the same method.

If the value obtained by the Folin-Marenzi method is the true one, this method must be reacting to a desulphurized changed cystine left in the gastrointestinal tract, a complex to which the other methods do not react. If this assumption is made that the excess of material reacting to the Folin-Marenzi method is only changed cystine, then the findings by the Folin-Marenzi method are the true rate of absorption of cystine by the white rat.

Data are given to show that hydrolysates of amino acids with no cystine present react positively as cystine in the Folin-Marenzi method, while negative in the other two methods, and that feeding glycine, and especially alanine, gives an increase in cystine value by the Folin-Marenzi method.

Indications were obtained of an increase in glutathione content of the liver by feeding cystine.

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